ORIGINAL ARTICLE

Strong Association Between the –308 TNF Promoter Polymorphism and Allergic Rhinitis in Pakistani Patients

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Abstract

Background: Allergic rhinitis (AR) is a common complex allergic inflammatory disorder. The tumor necrosis factor (TNF) α polymorphism G–308A is present in the promoter region of the gene and considered to be important due to its role in different allergic diseases.
Objectives: We aimed to identify genetic associations between this polymorphism and AR in patients of Pakistani origin.
Methods: We analyzed the distribution of G–308A in 153 unrelated AR patients and 116 unrelated healthy controls. Samples were genotyped for G–308A using restriction fragment length polymorphism.
Results: The TNF –308A allele (TNF2) was significantly more frequent in patients with AR (37%) than in controls (16%, *P*<.001; χ^2=32.15). Genotype distribution was also significantly different between patients and controls (*P*<.001; χ^2=40.81). The TNF2 homozygous genotype and TNF1/2 heterozygous genotype were significantly more common in AR patients (TNF2, 12%; TNF1/2, 49%) than in controls (TNF2, 4%; TNF1/2, 23%), whereas the normal TNF1 homozygous genotype was more frequent in controls (73%) than in patients (39%).
Conclusion: Our results suggest a strong association between the promoter polymorphism of TNF-α and AR in Pakistani cohorts.

Key words: TNF-α. Allergic rhinitis. Polymorphism. Disease-association.

Resumen

Antecedentes: La rinitis alérgica (RA) es un trastorno alérgico inflamatorio complejo frecuente. El polimorfismo G-308A del factor de necrosis tumoral (TNF) α está presente en la región del promotor del gen y se considera importante debido a su función en diferentes enfermedades alérgicas.
Objetivos: El objetivo fue identificar las asociaciones genéticas entre este polimorfismo y la RA en pacientes de origen paquistaní.
Métodos: Se analizó la distribución de G-308A en 153 pacientes con RA no emparentados y 116 controles sanos no emparentados. Se determinó el genotipo G-308A de las muestras por medio de un polimorfismo de la longitud de los fragmentos de restricción.
Resultados: El alelo 308A del TNF (TNF2) fue significativamente más frecuente en los pacientes con RA (37%) que en los controles (16%, *p*<0,001; χ^2=32,15). La distribución del genotipo también fue significativamente diferente entre los pacientes y los controles (*p* < 0,001; χ^2=40,81). Los alelos TNF2 en homocigosis y TNF1/2 en heterocigosis se asociaron significativamente en mayor proporción con los pacientes con RA (TNF2, 12%; TNF1/2, 49%) que con los controles (TNF2, 4%; TNF1/2, 23%), mientras que el genotipo homocigótico TNF1 normal fue más frecuente en los controles (73%) que en los pacientes (39%).
Conclusión: Los resultados indican una estrecha relación entre el polimorfismo del promotor del TNF-α y la RA en cohortes de personas de origen paquistaní.

Palabras clave: TNF-α. Rinitis alérgica. Polimorfismo. Asociación de enfermedades.

Introduction

Allergic rhinitis (AR) is a common disease of the upper airway. It affects children and adults, and its prevalence, morbidity, and mortality have increased considerably. The prevalence of AR in Asia is estimated to be as high as 10%-30% in adults and 10%-46% in children [1]. In England and the USA, 3.3 million people are affected [2].

AR is a multicomplex inflammatory disorder of the nasal mucosa and occurs when inhaled allergens interact with immunoglobulin (Ig) E in cells in the airway [3]. Proinflammatory cytokines have been suggested to be involved in the upregulation of adhesion processes in human nasal mucosa and the activation of various cell populations involved in allergic inflammation. Cytokines, which originate in mast cells, eosinophils, and T lymphocytes, contribute to the underlying inflammatory process in rhinitis, and their levels are increased following local allergen challenge [4,5].

Tumor necrosis factor (TNF) α is a pleiotropic proinflammatory cytokine, high levels of which have been observed in pulmonary diseases [6]. It is found in nasal mucosal mast and epithelial cells [7], and augmented expression on the cytokine of TNF receptors has been reported in patients with AR [8]. Iwasaki et al [9] observed that lack of TNF-α inhibited development of AR in mice. These data suggest that TNF-α may play a role in the pathogenesis of AR.

Several known polymorphisms have been reported in the promoter region of the TNF-α gene, among which the well-defined biallelic polymorphism at position –308 comprises a common variant with a guanine (G) at position –308 (TNF1) and an uncommon variant with an adenine (A) at –308 (TNF2). Although still controversial, most available data support a direct role for this polymorphism when TNF-α levels are high. Elevated TNF-α level due to the TNF2 allele has been shown to directly affect TNF-α expression and may alter the immune response in such a way that it confers susceptibility to certain autoimmune diseases, infectious diseases, and immune-mediated disorders [10-12]. However, data related to the association between G–308A and AR remain limited. Zhu et al [13] did not find an association between the TNF2 allele and AR. In contrast, Gentile et al [12] reported high frequencies of the TNF–α genotype in infants with a parental history of AR and suggested a role for the high production of TNF-α in the pathogenesis of the disease. Apart from these 2 studies, no authors to date have determined an association between this polymorphism and AR to date. Therefore, we performed the present study to determine the association between G–308A and AR in Pakistani cohorts.

Patients and Methods

This study was approved by the ethics committee of the Department of Biosciences, COMSATS Institute of Information Technology and Allergy Center. Patients were recruited during July 2007-October 2007 from the Allergy Centre, National Institute of Health, Islamabad, Pakistan. Diagnosis was based on nasal obstruction, postnasal drip, sneezing, and positive reaction to allergen extracts and IgE levels in a skin prick test (SPT). Control groups were not positive to allergen extracts or had no positive family history of allergy. Differences in mean (SD) total IgE levels were observed to be statistically significant ($P>.0001$) between the controls (1.95 [0.05] IU/mL) and patients (2.28 [0.04] IU/mL).

Blood was extracted from each participant after obtaining informed consent. Genomic DNA was extracted from the 4 mL of peripheral blood leukocytes using the organic (phenol-chloroform) method. Genotyping for G–308A was performed using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism. The TNF-α polymorphism was amplified using an upstream primer with a mismatch that introduced an artificial Sty 1 restriction site into the wild-type allele (allele 1), but not in the variant allele (allele 2). The forward primer for the –308 polymorphism used was 5'AGGCAATAGGTTTTGAGGGCCATG' and the 3' reverse primer was '5-ACACACAAGCATCAAGGATACC-3'. A 143-bp fragment was amplified with this primer set. Each 25-mL PCR reaction contained 2.5 μL of 10X PCR buffer, 1.5 mMol of $MgCl_2$, 10 pmol of each primer, 0.2 mM of the dNTPs, 15 μL of deionized water, 1 μL of Taq DNA polymerase, and 40-50 ng of genomic DNA as a template. The mixture was denatured at 95ºC for 5 minutes and underwent 35 cycles in a thermocycler PCR system under the following conditions: denaturation at 95ºC for 1 minute, annealing at 60ºC for 45 seconds, extension at 72ºC for 1 minute, and a final extension for 10 minutes at 72ºC. The amplified fragments were detected on 2% agarose gel (Invitrogen, Carlsbad, California, USA). Restriction enzyme digestion of PCR products was carried out by adding 2 μL of Buffer O and 1 μL (10U) of restriction enzyme Sty 1 (Fermentas, Hanover, Maryland, USA). Digested fragments were observed on 3% agarose gels.

Statistical analysis was performed using SPSS version 16 (SPSS Inc, Chicago, Illinois, USA). AR and control data were compared using the χ^2 test and Fisher exact test. Statistical significance was set at $P<.05$.

Results

To assess the association with G–308A in the study population, we compared 153 AR patients (mean age, 30.6 [10.52] y) with 116 healthy controls (mean age, 32.95 [9.95] y). The means were not significantly different between the 2 groups ($P=.2$). The percentage distribution of gender was also equal in both groups (70% men and 30% women).

The G–308A genotype and allele frequencies were in Hardy–Weinberg equilibrium in both the patients and the controls ($\chi^2=2.44$; $P=.1$). Table 1 shows the allelic frequency distribution in patients and controls. Table 2 shows the genotype frequency distribution of G–308A. Analysis of allelic frequency indicated TNF2 to be more significantly present in patients with AR than controls and associated with the disease (Table 1). The difference in genotype frequency distribution was also found to be extremely significant in the patients (TNF1, 39%; TNF1/2, 49%; and TNF2, 12%) when compared with the controls (TNF1, 73%; TNF1/2, 23%; and TNF2, 4%) (Table 2).

Table 1. Allelic Frequencies of TNF–α G-308A Polymorphism in Controls and Allergic Rhinitis Patients

Allele	Controls	Patients	Controls	Patients
GG (TNF1)	196 (84%)	193 (63%)	<.001 (30.21)	3.01(2.04-4.99)
A (TNF2)	36 (16%)	113 (37%)		

Table 2. Genotypic Frequencies of TNF-α G–308A Polymorphism in Controls and Allergic Rhinitis Patients

Genotypes	Controls (n=116)	Patients (n=153)	*P* Value (χ^2)	Odds Ratio	*P* Value (χ^2)
GG (TNF1)	85 (73%)	59 (39%)	<.001 (31.96)	4.37 (2.5-7.65)	
GA (TNF1/2)	26 (23%)	75 (49%)	<.001 (19.92)	0.30 (0.17-0.53)	<.001 (32.15)
AA (TNF2)	5 (4%)	19 (12%)	.02 (5.34)	0.32 (0.10-0.94)	

Discussion

We found that patients carrying the TNF2 allele of the TNF-α –308 promoter region more commonly had AR. To the best of our knowledge, this is the first report of such an extreme association between the TNF2 allele and AR. Previously, Gentile et al [12] concluded that this genotype was significantly associated with increased production of TNF-α and AR in infants. In contrast, Zhu et al [13] did not find any association between this polymorphism and AR.

The proinflammatory cytokine TNF-α can mediate nasal allergic inflammation [14]. It has been suggested that TNF-α may be involved in the upregulation of expression of endothelial cell adhesion molecules and activation of various cell populations in human nasal mucosa during allergic inflammation [5]. Several studies have indicated that the –308 promoter region of the TNF-α gene is critical for the determination of TNF-α expression levels. The TNF2 allele has been shown to be associated with higher circulating levels of TNF-α and the TNF1 allele is a comparative low secretor allele [15]. We found the frequency of the TNF2 allele to be significantly more frequent in patients (37%) than in healthy controls (16%; χ^2=30.21; *P*<.001), suggesting an increase in TNF-α expression levels in AR. Expression of the –308 polymorphism is not only based on the allele type, but is also dependent on cell type and stimulus specificity [16]. Bradding et al [7] provided evidence of mast cells as an important source of TNF–α in patients with AR. Some authors have observed significant upregulation of TNF-α levels in nasal lavage fluid following allergen challenge in patients with AR [5,7], and increased expression levels of TNF receptor on nasal epithelial cells from patients with AR have been reported [17]. Lack of TNF-α has also been observed to inhibit the development of AR in mice [9]. Tanabe et al [18] demonstrated activation of TNF-α production following addition of pollen to peripheral blood mononuclear cells. Moreover, anti-TNF-antibody therapy has also been reported to inhibit IgE-mediated allergy [19]. These findings support the involvement of the TNF2 allele in AR, as observed in our study. However, this allele has also been significantly linked with the high TNF-α–producing autoimmune major histocompatibility complex HLA A1-B8-DR3 haplotype [20]. The extended haplotype (HLA B8-SC01-DR3) and DR3 is more common among patients with rhinitis than in controls [21]. We propose the possibility of a gene–gene interaction associated with AR.

Our genotype distribution data also support the significance of the TNF2 allele in our study population and show that the TNF2 homozygote is significantly associated with AR. The most common genotype in AR patients was the TNF1/2 heterozygote, which was found to be strongly associated with the disease (odds ratio, 19.92; *P*<.001).

In conclusion, we report an extremely significant association between the TNF-α –308 promoter region polymorphism and AR in Pakistani patients. Extension of the study of the TNF polymorphism to associations with MHC haplotypes and phenotypic expression will help to better define our findings.

Acknowledgments

The authors would like to acknowledge the technical staff at the Allergy Centre, National Institute of Health, Islamabad, Pakistan for assistance with patient recruitment.

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Manuscript received February 15, 2010; accepted for publication March 14, 2010.

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